# Kinetics of the Decomposition of Coprohaem Compound I in Aqueous Solution

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Coprohaem Compound I, formed by oxidation of coproferrihaem {[3,8,13,17-tetramethylporphyrin-2,7,12,18-tetrapropionato(6−)-$N^{21}$,$N^{22}$,$N^{23}$,$N^{24}$]ferrate(3−)} with peroxyacids in reactions of 1:1 stoicheiometry, decomposes in aqueous solution in the absence of added reductants. The reaction is biphasic and follows series first-order kinetics. The formation of a coprohaem Compound II intermediate has been implicated. When the initial ratio of [peroxyacid]:[coproferrihaem] was $\leqslant 1$, an approximately constant proportion (75–80%) of the Compound I initially formed was reduced back to coproferrihaem and the remainder was converted into products which were non-absorbing in the Soret and visible spectral regions. It is proposed that coproferrihaem Compound I undergoes a rate-determining reaction with water, probably involving nucleophilic attack at the porphyrin meso positions, to form species which are susceptible to rapid oxidative attack by other Compound I molecules and resulting in the reduction of 3—4 molecules of Compound I for each molecule of Compound I oxidatively destroyed.

The ferrihaem hydroperoxidases of sub-group E.C.1.11.1 (peroxidases and catalases) react with hydroperoxides to form oxidised derivatives, the Compound I intermediates,[1] which retain both oxidising equivalents of the hydroperoxide progenitor and are, therefore, formally '$Fe^{V}$' species. In most (but not all) cases, the oxidising equivalents are located on the haem moiety of enzymes and these species have been characterised as ferryl–porphyrin π-cation radical complexes.[2–4] Under normal catalytic conditions reduction of Compound I to the native, $Fe^{III}$, enzyme by appropriate donor molecules completes the enzymatic cycle.

In the absence of deliberately added donor, the enzyme Compound I species are not indefinitely stable and they decompose with reformation of a functional enzyme that may be essentially complete.[5] The origin of the reducing equivalents which bring about this decay has been discussed but by no means completely resolved.[6] A clear distinction between reduction associated with impurities (adventitious donors) and reduction which is an intrinsic feature of Compound I chemistry, involving endogenous donor(s), is difficult to make experimentally. Endogenous donors have been suggested to be protein residues,[7] in which case the Compound I decay process does not yield the native protein but a modified protein which may nonetheless retain functional capacity. Kinetically, the decay of Compound I to the native enzyme is a biphasic process involving consecutive one-electron reduction steps and the intermediacy of $Fe^{IV}$, Compound II, enzyme species.[5]

Compound I derivatives have been formed by the oxidation of protein-free, monomeric ferrihaem complexes in non-aqueous solvents such as dichloromethane.[8] Under these conditions the intermediates are very stable in the absence of added donor but are markedly destabilized by the addition of water to the system. The possible development of photochemical energy conversion devices has stimulated extensive studies of porphyrin π-cation radicals and their complexes with non-redox active metal ions in aqueous solutions.[9] Both the (desirable) formation of oxygen by water oxidation and the (undesirable) porphyrin degradation processes that occur have been investigated.[10]

Although the formation of Compound I intermediates has been inferred in many studies of the peroxidase and catalase-like actions of ferrihaem complexes in aqueous solution,[11] the direct observation of a Compound I species under these conditions has been frustrated by a range of complicating factors. These problems have been resolved in the studies of the reaction of coproferrihaem (cfh) with peroxyacids described in the previous paper.[12] Coprohaem Compound I (ch-I) is a very efficient oxidant species towards a range of typical peroxidase donor substrates but, even in the absence of added donor substrate, undergoes rapid decomposition in aqueous solution at near ambient temperatures. The present paper reports studies of the nature and kinetics of this 'spontaneous' decomposition process.

## Experimental

*Materials.*—The sources and characterization of materials have been described previously.[12] Triply-distilled water was used for all solutions. Deuterium oxide as supplied (ICI, 99.99% D) exhibited appreciable u.v. absorbance and rapidly reduced peroxyacids. After treatment with *m*-chloroperoxybenzoic acid, distillation under nitrogen and further triple distillation employing deuteriated materials, the resulting $D_2O$ showed no significant u.v. absorbance and yielded stable peroxyacid solutions.

*Methods.*—Kinetic studies employed a Durrum D110 stopped-flow spectrophotometer interfaced to a transient recorder and microcomputer.[13] Studies with gas-saturated solutions employed a Hewlett Packard 8541 diode-array spectrophotometer with a stopped-flow attachment.

## Results and Discussion

The rapid, stoicheiometric formation of ch-I by reaction of cfh with peroxyacids,[12] cfh + ROOH ⟶ ch-I + ROH, is associated with a marked decrease in absorbance at the Soret band maximum ($\lambda$ = 390 nm) of cfh. For values of $R$ (the initial ratio $[\text{peroxyacid}]_0:[\text{cfh}]_0$) in the range 1—4, the maximum decrease in absorbance was independent of $R$. Subsequent to ch-I formation the absorbance increases again so that, after *ca.* 100 s at 25 °C the absorbance of the solution is time invariant and the absorption spectrum indicates the partial reformation of cfh (to an extent which decreases with increasing $R$), together with, implicitly, cfh degradation products which are non-absorbing. For $R > 6$ the reaction leads to final solutions which are non-absorbing in the Soret and visible wavelength regions.

When $R \leqslant 1$ the absorbance begins to increase immediately following ch-I formation. When $1 < R < 4$ (*i.e.* excess of peroxyacid over that required for stoicheiometric formation of ch-I) the absorbance decrement remains near the maximum value for a period which increases with increasing $R$ before a rapid increase in absorbance ensues. In this 'quasi-steady-state' region it is likely that a complex series of reactions can occur, including catalytic turnover of peroxyacid and haem degradation by direct attack of excess peroxyacid upon iron–porphyrin species or their initial degradation products. For this reason most of our experiments have been carried out with $R \leqslant 1$, to avoid complexity associated with the presence of excess peroxyacid in the system.

Since the formation of cfh from ch-I is a two-electron process the necessary reducing equivalents must be provided either by species present in the system at the point of maximal ch-I formation or formed continuously in events subsequent to ch-I formation. We have attempted to characterise: (*i*) the kinetics of the ch-I decomposition process and (*ii*) the relationship between the formation of cfh and haem destruction products in the process.

The time course of the change in absorbance associated with the decay of ch-I is biphasic and well described as a series first-order process [equation (1)]. When a reaction occurring by such

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \qquad (1)$$

a process is monitored spectrophotometrically at fixed wavelength the optical density, $D$, of the solution at time $t$ is given by equation (2) provided that the species involved obey

$$D = E_A[A]l + E_B[B]l + E_C[C]l \qquad (2)$$

Beer's law, where $E_A$, $E_B$, and $E_C$ are the absorption coefficients of A, B, and C respectively and $l$ is the cell pathlength. The time variation of $D$, obtained by introducing standard expressions for the time dependence of [A], [B], and [C], may be resolved into three linear ($a_0$, $a_1$, $a_2$) and two non-linear terms, equation (3). From appropriate optical density *versus* time data the rate

$$D = a_0 + a_1 \exp(-k_1 t) + a_2 \exp(-k_2 t) \qquad (3)$$

constants, $k_1$ and $k_2$, can be extracted in various ways. Graphical analysis is straightforward, as has been described *e.g.*[5] for the decay of chloroperoxidase Compound I, and has been employed in the present study to obtain initial estimates of the rate constants. For more detailed analysis the non-linear least-squares ELORMA routine[14] was employed. In this method only the non-linear parameters (rate constants) are successively approximated; linear parameters are calculated only once using the final set of non-linear estimates. Elimination of the linear parameters[15] means that ELORMA requires initial estimates only of the rate constants.

As Alcock *et al.*[16] have pointed out, the analysis of series first-order kinetics from optical density data yields two mathematical solutions corresponding to interchange of the rate constants with a corresponding adjustment of the absorption coefficient of species B. The implications of this ambiguity in the present context are discussed later.

Values of $k_1$ and $k_2$ obtained by this procedure for various conditions are shown in Table 1. Unless otherwise indicated the experiments were carried out at pH = 6.0 (10 mmol dm$^{-3}$ phosphate buffer), $I$ = 0.1 mol dm$^{-3}$ (adjusted with NaCl), and 25 °C. At the concentrations of cfh employed, >99% cfh is present as monomeric ferrihaem.[17] The rate constants were independent of wavelength through the Soret region and most studies were carried out at 390 nm (the Soret band maximum of cfh) where absorbancy changes were maximal. The results are summarized as follows: (*a*) $k_1$ and $k_2$ were independent of [cfh]$_0$ at $R = 1$ and hence independent of [ch-I]$_0$, (*b*) the rate constants were independent of the initial presence of excess unoxidised cfh in the solution, (*c*) the rate constants were

**Table 1.** Kinetic data for the decomposition of coprohaem Compound I. Unless otherwise indicated the following experimental conditions were employed: [cfh]$_0$ = 1.0 μmol dm$^{-3}$, $R$ = 1.0 (oxidant *m*-chloroperoxybenzoic acid), pH = 6.0 (10 mmol dm$^{-3}$ phosphate buffer), $I$ = 0.1 mol dm$^{-3}$ (adjusted with NaCl), 25 °C, measuring wavelength λ = 390 nm

| $10^2 k_1/s^{-1}$ | $10^3 k_2/s^{-1}$ | |
|---|---|---|
| (*a*) Variation of measuring wavelength | | |
| | | λ/nm |
| 53 ± 4 | 65 ± 4 | 380 |
| 54 ± 2 | 67 ± 2 | 385 |
| 56 ± 5 | 72 ± 5 | 390 |
| 52 ± 2 | 59 ± 15 | 395 |
| (*b*) Variation of [cfh]$_0$ and $R$ | | |
| | | [cfh]$_0$/μmol dm$^{-3}$ / $R$ |
| 56 ± 3 | 70 ± 4 | 1.5 / 0.5 |
| 56 ± 1 | 71 ± 3 | 1.5 / 1.0 |
| 62 ± 4 | 74 ± 3 | 3.0 / 0.5 |
| 64 ± 5 | 75 ± 3 | 3.0 / 1.0 |
| 57 ± 5 | 74 ± 3 | 4.5 / 0.5 |
| 64 ± 6 | 88 ± 2 | 4.5 / 1.0 |
| 75 ± 6 | 73 ± 1 | 6.0 / 0.5 |
| | 71 ± 1 | 6.0 / 1.0 |
| (*c*) Variation of peroxyacid | | |
| | | Peroxyacid |
| 54 ± 2 | 70 ± 4 | *o*-chloroperoxybenzoic |
| 52 ± 1 | 69 ± 3 | *p*-chloroperoxybenzoic |
| 57 ± 2 | 74 ± 2 | *m*-nitroperoxybenzoic |
| 54 ± 2 | 71 ± 6 | *p*-nitroperoxybenzoic |
| 52 ± 4 | 66 ± 11 | *p*-methylperoxybenzoic |
| (*d*) Variation of phosphate buffer concentration and salt used for ionic strength adjustment | | |
| | | [buffer]/mmol dm$^{-3}$ / $I$ Adjustment |
| 46 ± 2 | 56 ± 1 | 1.0 |
| 45 ± 2 | 56 ± 1 | 1.0 $NaNO_3$ |
| 50 ± 2 | 65 ± 1 | 5.0 |
| 50 ± 1 | 62 ± 1 | 5.0 $NaNO_3$ |
| 55 ± 1 | 71 ± 3 | 10.0 |
| 55 ± 1 | 71 ± 5 | 10.0 $NaNO_3$ |
| 59 ± 2 | 72 ± 4 | 20.0 |
| 59 ± 1 | 73 ± 2 | 20.0 $NaNO_3$ |
| 65 ± 2 | 76 ± 3 | 50.0 |
| 67 ± 2 | 79 ± 4 | 50.0 $NaNO_3$ |
| (*e*) Effects of added *m*-chlorobenzoic acid | | |
| | | [*m*-$ClC_6H_4CO_2H$]/mmol dm$^{-3}$ |
| 56 ± 2 | 76 ± 3 | 0.00 |
| 54 ± 1 | 73 ± 4 | 0.25 |
| 57 ± 3 | 77 ± 7 | 0.50 |
| 56 ± 1 | 71 ± 3 | 1.00 |
| 57 ± 2 | 74 ± 2 | 1.50 |
| (*f*) Comparison of reaction in $H_2O$ and $D_2O$ | | |
| | | Solvent |
| 64 ± 3 | 88 ± 2 | $D_2O$, pD = 6.0 |
| 55 ± 3 | 71 ± 5 | $H_2O$, pH = 6.0 |

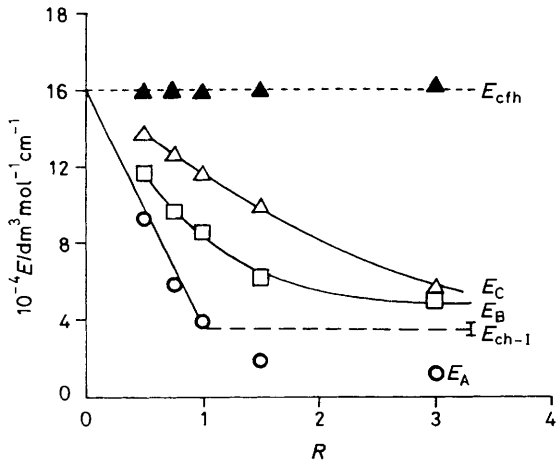


**Figure 1.** Example of the variation of ELORMA estimated absorption coefficients with $R$. Experimental conditions: $[cfh]_0 = 1.0$ μmol dm$^{-3}$, oxidant *m*-nitroperoxybenzoic acid, pH = 6.0 (10 mmol dm$^{-3}$ phosphate buffer), $I = 0.1$ mol dm$^{-3}$ (NaCl), 25 °C, measuring wavelength $\lambda = 390$ nm. Absorption coefficients: $E_A$ (○), solid line represents variation of $E_A$ with $R$ at $R \leqslant 1$ according to equation (4); $E_B$ (□); $E_C$ (△). Filled triangles show values of $E_C$ divided by the fraction of the initial cfh absorbance remaining at the end of reaction

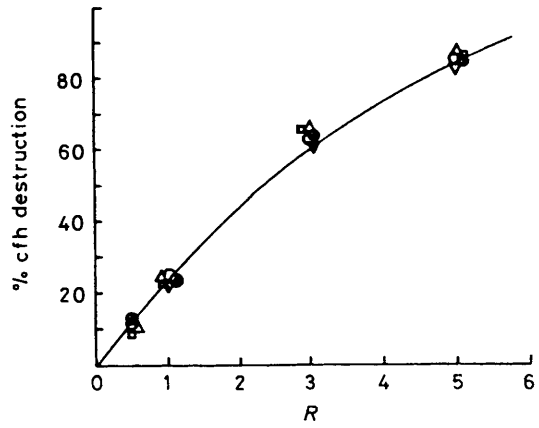


**Figure 2.** Variation of % destruction with $R$ at different phosphate buffer concentrations: [phosphate buffer] = 1 (○), 5 (◑), 10 (△), 20 (▽), and 50 mmol dm$^{-3}$ (□). Experimental conditions: $[cfh]_0 = 1.0$ μmol dm$^{-3}$, oxidant *m*-chloroperoxybenzoic acid, pH = 6.0, $I = 0.1$ mol dm$^{-3}$, 25 °C, $\lambda = 390$ nm

independent of the nature and concentration of peroxyacid used in the oxidation of cfh to ch-I for a series of peroxybenzoic acids, (*d*) adjustment of ionic strength with $NaNO_3$ rather than NaCl had no effect, (*e*) increase in phosphate buffer concentration at fixed pH and ionic strength resulted in a systematic increase in both $k_1$ and $k_2$ (this effect is rather small, *i.e.* a 50-fold increase in buffer concentration from 1 to 50 mmol dm$^{-3}$ produced a 40% increase in rate constants), (*f*) values of $k_1$ and $k_2$ in $D_2O$ at pD = 6.0 were *ca.* 20% higher than the corresponding values in $H_2O$ at pH = 6.0, and (*g*) the initial presence of excess *m*-chlorobenzoic acid (*i.e.* the reduction product of *m*-chloroperoxybenzoic acid) had no effect on the rate constants.

The ELORMA estimated values of the absorption coefficient $E_C$ decrease with increasing $R$ in a manner consistent with the presence in the final solution of non-absorbing haem destruction products (Figure 1). The values of $E_A$ also decrease with increasing $R$. At $R \leqslant 1$ this is expected since the initial system comprises a mixture of cfh and ch-I in proportions determined by $R$. At $R \leqslant 1$ the values are in accord with equation (4). At

$$E_A = RE_{ch\text{-}I} + (1-R)E_{cfh} \qquad (4)$$

$R > 1$ the values of $E_A$ do not remain constant at $E_A = E_{CH\text{-}I}$ but continue to decrease with increasing $R$. This behaviour suggests that, at $R > 1$, the presence of excess peroxyacid during the 'quasi-steady-state' period results in the formation of haem destruction products. The present discussion will be restricted to $R \leqslant 1$.

Values of $E_B$ also decrease with increasing $R$. At $R = 1$ the data yield $E_B = 8.8 \times 10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$ at $\lambda = 390$ nm for the solution $k_1 > k_2$. The alternative solution ($k_2 > k_1$) yields $E_B = 4.2 \times 10^5$ dm$^3$ mol$^{-1}$ cm$^{-1}$ at $\lambda = 390$ nm. The latter value is improbably high for an iron–porphyrin species or derivative and it is thus reasonable to accept the solution $k_1 > k_2$ for the system. If haem destruction is totally associated with the transition A ⟶ B then the value of $E_B$ would be increased to *ca.* $12 \times 10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$ ($k_1 > k_2$) at 390 nm. Studies at a number of wavelengths in the Soret region indicate that $E_B$ shows a Soret band maximum near that of cfh itself. Although the values of $E_B$ are subject to uncertainty associated with the occurrence of haem destruction, the results are consistent with the concept that B is an analogue of enzymatic Compound II intermediates so that the decomposition of ch-I may be represented as shown below. As indicated a corollary of

$$\text{ch-I} \xrightarrow[+e]{k_1} \text{ch-II} \xrightarrow[+e]{k_2} \text{cfh}$$
$$\underbrace{\qquad\qquad}_{+\text{ Destruction products}}$$

this formulation is that the reduction of ch-I to cfh occurs *via* two consecutive one-electron reduction processes and the origin of these reducing equivalents must therefore be addressed.

It is unlikely that significant reducing equivalents are provided by adventitious impurities in the cfh preparations since the kinetics of ch-I decomposition are unaffected in the presence of an initial excess of cfh (and hence an increased concentration of hypothetical reducing impurity). Ch-I decomposition kinetics are unaffected by the initial presence of excess *m*-chlorobenzoic acid so that direct oxidation of benzoic acid, present as the reduction product of peroxycarboxylic acid, cannot contribute significantly to ch-I reduction. The increased rate constants with increasing phosphate buffer concentration could have several explanations, including the possibility of reducing impurities in the buffer components. However, the small effect of a large increase in buffer concentration suggests that, if this is indeed an impurity effect, it is not of major significance. Since the extent of haem destruction is unaffected by changing buffer concentration (Figure 2) under otherwise identical conditions it is more probable that change in buffer concentration exerts a direct medium effect on $k_1$ and $k_2$.

If a parallel reaction to ch-I formation can occur between cfh and peroxyacid to yield an oxidation susceptible species, $X_1$, it is possible that, although, at $R \leqslant 1$, $X_1$ may be formed at concentrations low enough to escape spectrophotometric detection, it may provide sufficient reducing equivalents to bring about substantial regeneration of cfh (see below). Such a

$$\text{cfh} + \text{ROOH} \begin{cases} \longrightarrow \text{ch-I} \\ \longrightarrow X_1 \end{cases}$$
$$\text{ch-I} + X_1 \longrightarrow X_2 + \text{cfh}$$
$$\text{ch-I} + X_2 \longrightarrow X_3 + \text{cfh}$$
$$\textit{etc.}$$

**Table 2.** Influence of reaction conditions on the destruction of the cfh chromophore associated with the formation and decomposition of ch-I. % Destruction = $[(D_{390})_0 - (D_{390})_x]/(D_{390})_0 \times 100$. Experimental conditions: pH = 6.0 (10 mol $dm^{-3}$ phosphate buffer), $I$ = 0.1 mol $dm^{-3}$ (adjusted with NaCl), oxidant *m*-chloroperoxybenzoic acid, measuring wavelength $\lambda$ = 390 nm

| $[cfh]_0$/ mol $dm^{-3}$ | $\theta_c$/°C | % Destruction $R$ = 0.5 | $R$ = 1.0 | $R$ = 2.0 | $R$ = 3.0 |
|---|---|---|---|---|---|
| 1.5 | 25 | 10.9 | 23.3 | 43.6 | |
| 3.0 | 25 | 10.0 | 23.1 | 43.4 | |
| 4.5 | 25 | 10.8 | 22.4 | 40.3 | |
| 6.0 | 25 | 9.4 | 21.7 | 38.7 | |
| 1.0 | 25 | | 20.4 | 42.9 | |
| 1.0 | 14.2 | | 19.5 | 38.6 | |
| 1.0 | 5.0 | | 20.8 | 40.2 | |
| 3.0[a] | 25 | | 24.9 | | 56.5 |
| 3.0[b] | 25 | | 24.5 | | 55.8 |

[a] Solutions saturated with $N_2$. [b] Solutions saturated with $O_2$.

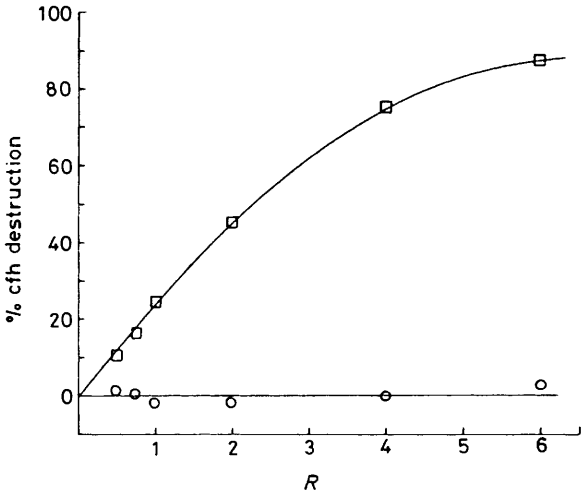


**Figure 3.** Effects of added ascorbic acid on the variation of % destruction with $R$. Experimental conditions: $[cfh]_0$ = 1.0 μmol $dm^{-3}$, oxidant *m*-chloroperoxybenzoic acid, pH = 6.0 (10 mmol $dm^{-3}$ phosphate buffer), $I$ = 0.1 mol $dm^{-3}$, 25 °C, $\lambda$ = 390 nm. No ascorbic acid added (□); 50 μmol $dm^{-3}$ ascorbic acid added (○)

scheme would be expected to yield a second-order decay of ch-I at $R \leqslant 1$. Moreover, the following experiments imply that this cannot be a significant pathway. Ch-I is very rapidly reduced to cfh by ascorbate so that, if the reaction of cfh with peroxyacid is carried out in the presence of ascorbate the cfh absorbance will change if direct reaction to form $X_1$ occurs. If, on the other hand, oxidisable species are formed only from ch-I itself, the cfh absorbance should remain constant, even at high $R$, when ch-I is efficiently scavenged by ascorbate. The results, shown in Figure 3, indicate that the latter expectation is fulfilled, even at $R$ = 6 where, in the absence of ascorbate, *ca.* 90% of the initial cfh absorbance is lost.

Overall, the results imply that the reduction of ch-I to cfh occurs at the expense of the oxidation of a proportion of the ch-I to non-absorbing haem destruction products. The extent of haem destruction (Table 2) under otherwise identical conditions is unaffected by (*a*) variation of buffer concentration (Figure 2), (*b*) variation of the peroxyacid oxidant employed (Figure 4), and (*c*) initial addition of excess carboxylic acid to the solutions (Figure 5). At $R \leqslant 1$ an approximately constant proportion of

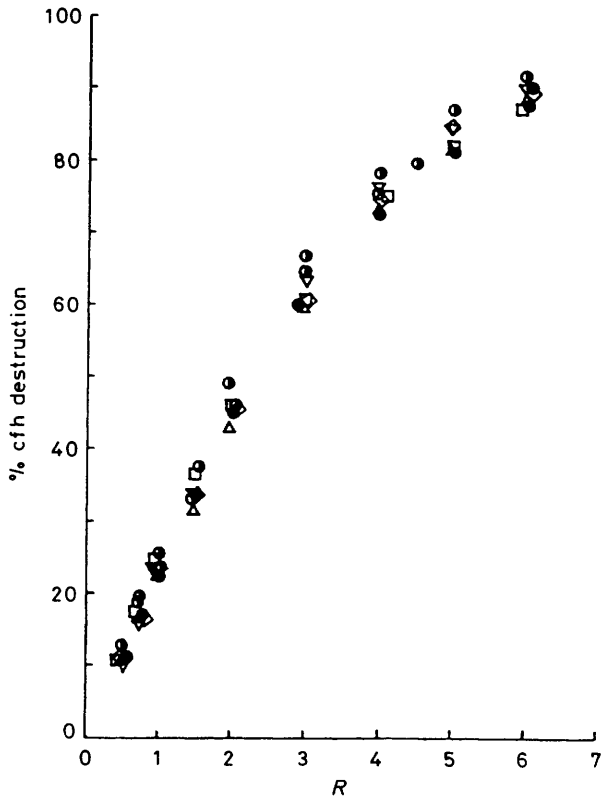


**Figure 4.** Variation of % destruction with $R$ for a range of peroxybenzoic acid oxidants: *m*-nitro- (○), *m*-chloro- (□), *o*-chloro- (△), *p*-chloro- (◇), *p*-nitro- (●), and *p*-methyl-peroxybenzoic acid (▽). Other experimental conditions: $[cfh]_0$ = 1.0 μmol $dm^{-3}$, pH = 6.0 (10 mmol $dm^{-3}$ phosphate buffer), $I$ = 0.1 mol $dm^{-3}$, 25 °C, $\lambda$ = 390 nm

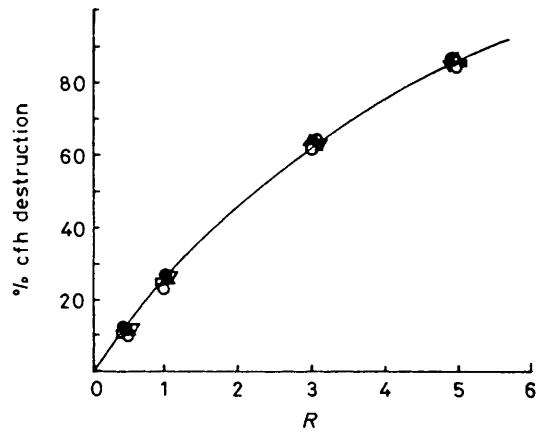


**Figure 5.** Effects of added *m*-chlorobenzoic acid on the variation of % destruction with $R$. Oxidant was *m*-chloroperoxybenzoic acid; other experimental conditions as in Figure 4. [*m*-chlorobenzoic acid] added: 0 (○), 0.25 (▲), 0.50 (□), 1.00 (▽), and 1.50 mmol $dm^{-3}$ (◐)

the ch-I is destroyed (20—25%), corresponding to the reduction of 3—4 molecules of ch-I to cfh for each molecule of ch-I that undergoes destruction. The kinetics suggest that, rather than this process being initiated by a direct bimolecular reaction between ch-I molecules, a rate-determining (r.d.) first-order activation of ch-I to form oxidisable species occurs. There are good grounds for believing that this process involves attack of solvent upon ch-I.

The absorption spectrum of ch-I is consistent with a ferryl–porphyrin π-cation radical Compound I species. The decay of metalloporphyrin π-cation radicals generated by pulse radiolysis has been extensively studied.[18–20] An important pathway involves bimolecular disproportionation to yield metalloporphyrins and the metalloporphyrin di-cation complex. The latter species is a powerful electrophile which reacts with water to generate isoporphyrins and meso hydroxy porphyrin species which can participate in further degradation processes.[21] In this concept regeneration and destruction of porphyrin are closely integrated. However, disproportionation of ch-I is not a tenable interpretation since the decomposition kinetics are not second order.

There is increasing evidence that porphyrin π-cation radicals are themselves susceptible to nucleophilic attack at the meso positions by water or hydroxide ion generating isoporphyrins and meso hydroxy porphyrins;[22] indeed this route has been used to synthesize meso-substituted porphyrins.[23] The concept that ch-I is activated by a rate-determining nucleophile attack by solvent for a rapid cascade of oxidation by other ch-I molecules is consistent with the present results, *i.e.* equations (5) and (6) *etc.* The results also imply that reduction of ch-I

$$\text{ch-I} + H_2O \xrightarrow{\text{r.d.}} Y_1 \qquad (5)$$

$$\text{ch-I} + Y_1 \xrightarrow{\text{fast}} Y_2 \qquad (6)$$

proceeds by one-electron steps, forming ch-II as an intermediate which can initiate further reaction although at a much reduced rate. The concept of a single rate-limiting step in a process which leads both to regeneration of cfh and the formation of haem destruction products is supported by the observation that the fraction of cfh destroyed is independent of temperature under otherwise comparable conditions (Table 2). Since radicals formed in one-electron redox processes might engage in autoxidative degradation reactions with dissolved $O_2$, the extent of cfh destruction was also compared under anaerobic and oxygen-saturated conditions. No significant difference in the extent of cfh destruction was observed (Table 2).

The completely protective effect of ascorbate as a ch-I scavenger even at high $R$ suggests that increased haem destruction at high $R$ in the absence of scavenger results from attack of peroxyacid upon ch-I rather than upon cfh itself. Three additional pathways may be operative under these conditions: (*a*) regenerated cfh could be re-oxidised by peroxyacid to ch-I leading to further destruction *via* water attack; (*b*) nucleophilic attack by the powerful α-nucleophile peroxyanion at the meso-positions of the porphyrin π-cation radical may initiate a further type of oxidation cascade; and (*c*) catalytic turnover of peroxyacid, which has been observed[24] in the reaction of deuteroferrihaem with peroxyacetic acid and in the reaction of chloroperoxidase with *m*-chloroperoxybenzoic acid,[5] is a likely further complication.

Since the slope of the data in Figure 4 remains almost constant in the range $0 < R < 2$ it is likely that pathway (*a*) predominates when $1 < R < 2$. When $R > 2$ the curvature of Figure 4 decreases as would be expected if (*c*) becomes significant. The present data provide no support for significant reaction *via* pathway (*b*), although evidence that this type of reaction becomes important at high $R$ has been obtained with the deuteroferrihaem–peroxyacetic acid system[25] at higher pH.

The possibility that porphyrin π-cation radicals, formed by oxidation of photochemically generated porphyrin triplets, may oxidise water to oxygen has been extensively discussed in the context of attempts to develop artificial photosynthesis systems.[26] Although the overall four-electron oxidation of water by a porphyrin π-cation radical ($P^{\bullet+}$), equation (7), is

$$4P^{\bullet+} + 2H_2O \longrightarrow 4P + 4H^+ + O_2 \qquad (7)$$

exergonic its successful achievement is, as Grätzel[27] has pointed out, crucially dependent on the presence of a suitable heterogeneous catalyst. In the absence of an effective catalyst, the one-electron of water, equation (8), although endergonic [at

$$P^{\bullet+} + H_2O \longrightarrow P + H^+ + OH^{\bullet} \qquad (8)$$

pH = 7, $E^{0\prime} = +2.18$ V (for $OH^{\bullet}$–$H_2O$)[28] and reduction potentials for $P^{\bullet+}$ species and their metallocomplexes[28,29] lie in the range $E^{0\prime} = +0.9$ to $+1.8$ V] may be driven by efficient $OH^{\bullet}$ trapping to bring about porphyrin degradation. A one-electron oxidation of water by ch-I, equation (9), might sustain a

$$\text{ch-I} + H_2O \longrightarrow \text{ch-II} + H^+ + OH^{\bullet} \qquad (9)$$

flux of $OH^{\bullet}$ radicals comparable to the value of $k_1$ observed and a similar argument would hold for a slower ($k_2$) oxidation of water by ch-II. Hydroxyl radical would be trapped either by reaction with porphyrin or with other scavengers present in the system. Experiments using solutions containing *m*-chlorobenzoic acid yielded the result that neither the kinetics of ch-I decay (Table 1) nor the fraction of haem destroyed (Figure 5) were influenced by the presence of the benzoic acid, *i.e.* the benzoic acid is unable to trap $OH^{\bullet}$ even at [acid]:[porphyrin] ratios of $>10^3$. This result implies that $OH^{\bullet}$ *free* radical is not formed in the reaction, although it remains a possibility that an $OH^{\bullet}$ radical, bound to, or trapped within the solvent cage of the metalloporphyrin site at which it was formed is so efficiently scavenged by the ligand at its generation site that it is inaccessible to an external scavenger.

The instability of ch-I (and porphyrin π-cation radicals and their metallocomplexes generally) in water contrasts markedly with the stability of related species in non-aqueous solvents such as dichloromethane and it is therefore not surprising that the evolved design of haemoprotein hydroperoxidases reflects the desirability of excluding water from the haem site in the enzymes. Thus the ferrihaem prosthetic groups of native catalases[30] and horseradish peroxidase[31–33] do not possess an axially co-ordinated water molecule and it may be that the proteins of the enzymes provide mechanisms for the removal of the water product of hydrogen peroxide reduction from the active site. In contrast, yeast cytochrome c peroxidase, which does contain a haem-bound water molecule in the native enzyme,[34] forms a Compound I derivative which does not contain a porphyrin π-cation radical moiety.

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